SEROPREVALENCE OF HEPATITIS C IN DIABETIC PATIENTS IN AN URBAN SOUTH AFRICAN SETTING

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A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfillment of the requirements for the degree of Master of Medicine (MMED) in the branch of Internal Medicine

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DECLARATION

I, Manoko Elizabeth Seabi declare that this research report is my own work. It is being submitted for the degree of Master of Medicine in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

Signature……

Date :

DEDICATION

This is dedicated to my parents Mrs. M F Seabi and the late Mr. Machaba Livingstone Seabi and to my husband Hopewell Ntsinjana.

PRESENTATIONS ARISING FROM THIS RESEARCH

This work was presented at the South African Gastroenterology Society (SAGES) congress in Cape Town, South Africa on 8-11 August 2008.

ABSTRACT

INTRODUCTION: Both hepatitis C virus (HCV) infection and diabetes mellitus (DM) are common illnesses with potentially devastating complications. There have been studies suggesting that HCV is a potential cause of diabetes by initially causing insulin resistance which then progresses to overt DM.

STUDY DESIGN AND METHODS: Patients from the Diabetes Clinic at the Charlotte Maxeke Johannesburg Hospital were recruited to the study from January to November 2007. Both type 1 and type 2 diabetic patients were recruited and were compared to a group of first time, apparently healthy blood donors (data provided by the South African National Blood Services) for infection with HCV. Our patients were tested for HCV by the use of a rapid test device which detects the presence of HCV antibodies. Patients who were positive on antibody testing had a confirmatory PCR done.

RESULTS: The prevalence of HCV infection was higher in our diabetic group than in the blood donors (1, 55% vs. 0, 02%; *P*=0, 0001; Odds ratio 69; 95%CI; 22-212). Furthermore, the prevalence was higher amongst type 2 than in the type 1 diabetic patients but this difference was not statistically significant (7/443(1, 58%) vs. 1/73(1, 37%) *P*= >0, 05). Five of the eight

patients who had a positive antibody test were also positive on PCR testing, and this was still statistically significant (5/516 (0.97%) vs. 8/35194; *P* =0.005). Previous exposure to recognized risk factors for HCV infection did not appear to be related to acquiring HCV.

CONCLUSIONS: There appears to be a higher prevalence of HCV among patients with diabetes than in those without. Whether HCV has a direct causative role or not remains to be proven but there seems to be a link.

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TABLE OF CONTENTS

	Page
Declaration	ii
Dedication	iii
Publications and presentations	iv
Abstract	v
Acknowledgements	vii
Table of contents	viii
List of tables	xi
List of figures	xii
List of abbreviations	xiii
1.0 Introduction	1
1.1 Epidemiology	1
1.2 Modes of hepatitis C transmission	3
1.3 Hepatitis C virus types	3
1.4 Pathogenesis of hepatitis C related liver disease	4
1.5 Hepatitis C and diabetes	7
1.5.1 Effects on insulin signal transduction pathway	8
1.5.2 Effects on the pancreas	11
1.6 Prevalence of glucose abnormalities in patients with HCV	11

2. Motivation for the study 14

3. Materials and Methods 15

3.1 Study population 15

Age groups 15

Sampling method 15

Hepatitis C test device 15

Control group 16

3.2 Statistical analysis 17

3.3 Ethical clearance 18

4. Results

4.1 Clinical characteristics of the diabetic patients 19

4.2 Comparisons between diabetic patients who are anti HCV positive and negative 23

4.3 Comparisons between the diabetic patients and the blood donor group on antibody testing 24

4.4 Comparisons between the diabetic patients and the blood donors on HCV PCR 25

5. Discussion 26

6. Limitations of the study 31

7. Conclusions 32

8. Appendices 33

8.1 Research Protocol 33

8.2 Patient information and consent 40

8.3 Health Questionnaire 42

8.4 Ethics Clearance Certificate 44

9. References 45

LIST OF TABLES

Table	Page
Table 1: Characteristics of the diabetes patients	19
Table 2: Comparisons between the diabetic patients who are anti-HCV positive and negative	23
Table 3: Comparisons between the diabetic patients and the blood donor group on HCV antibody testing	24
Table 4: Comparisons between the diabetic patients and the blood donor group on HCV PCR	25

LIST OF FIGURES

Figure	Page
1. Global prevalence of Hepatitis C	2
2. HCV induced insulin resistance	10
3. Risk Factors for HCV in type 1 and type 2 DM patients	21

LIST OF ABBREVIATIONS

HCV- Hepatitis C virus

HIV- Human immune-deficiency virus

RNA- Ribonucleic acid

TNF- Tumour necrosis factor

IFN-Interferon

IRF- Interferon regulatory factor

TLR- Toll-like receptor

HBV-Hepatitis B virus

DM- Diabetes mellitus

IRS- Insulin receptor substrate

SOCS3- Suppressor of cytokine S3

HOMA- Homeostatis model assessment

UKPDS- United Kingdom Prospective study of diabetes

CMJA- Charlotte Maxeke Johannesburg Academic

SANBS- South African National blood services

T/F- Transfusion

HbA1c- Glycosylated haemoglobin

IV- Intravenous

IVDU- Intravenous drug use

HR- Hazard ratio

BMI- Body mass index

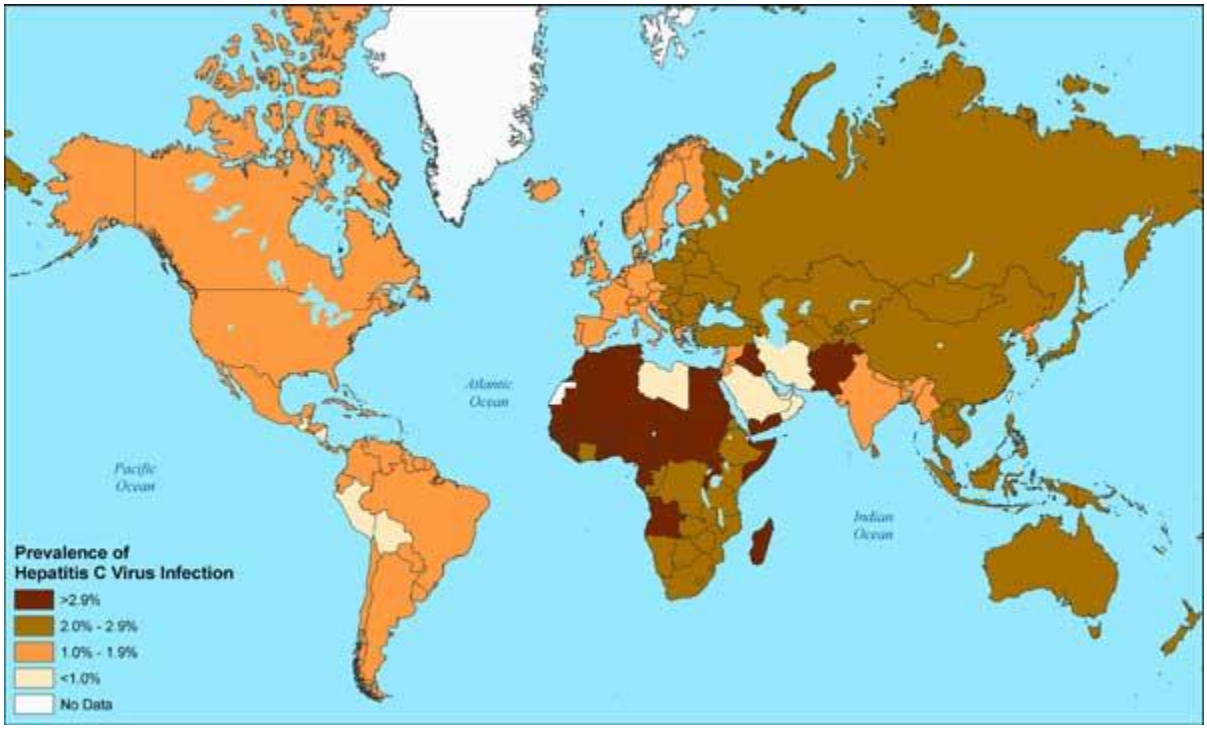
1. INTRODUCTION

1.1 Epidemiology

The rate of hepatitis C virus (HCV) infection has been gradually increasing over the years and currently an estimated 3% of the world's population is infected. Because of such high numbers of infection, HCV has been declared a global health problem [1]. The seroprevalence of HCV varies with different geographic locations with the highest reported prevalence rates in Africa and Asia and lower rates in North America, Western and Northern Europe and Australia [2] (See figure 1). The overall prevalence of HCV in Sub-Saharan Africa is estimated at 3%, with a higher prevalence in the central African region (6%) and lower rates in west Africa (2.4%) and southern and east Africa (1.6%)[3]. Egypt has an estimated population of 73 million and has the highest reported seroprevalence rate of 22% [3-4]. The prevalence of HCV in South Africa is low with an estimated 2.6% of the population infected [5].There is also a risk of co-infection with the human immunodeficiency virus (HIV) as some of the modes of transmission of these two viruses are similar. In countries such as India, rates of HCV/HIV co-infection vary according to route of transmission and

can range from 10-14% among persons reporting high risk sexual exposure to 85-90% among those reporting intravenous drug use [6, 33].

Figure 1: Global Prevalence of Hepatitis C



Modified form Perz JF, Farrington LA, Pecoraro C: Estimated global prevalence of Hepatitis C virus infection. 42nd Annual meeting of the Infectious Diseases Society of America; Boston, MA,USA; Sept 30-Oct 3, 2004. Data source: World Health Organization

1.2 Modes of Hepatitis C Transmission

HCV is transmitted by parenteral routes such as through the use of blood products (blood and immunoglobulin transfusions from unscreened donors), injectable drug use, contaminated medical equipment including razor blades used by traditional healers, tattoo equipment, and rarely sexually or perinatally [7]. Intravenous drug users have the highest prevalence of HCV infection, and the infection is thought to occur rapidly after initiating injecting drug behaviour [6, 40].

1.3 Hepatitis C virus Genotypes

HCV is a positive strand RNA virus and is the sole member of the hepacivirus genus, within the flaviviridae virus family [37, 38]. Six HCV genotypes and multiple sub-types (1a, 1b etc) have been identified on the basis of molecular relatedness and one patient may be infected with more than one genotype [8, 9]. Genotypes 1-3 have a world-wide distribution. Genotypes 1a and 1b are the most common, accounting for about 60% of global infections. In the United States and western Europe, genotypes 1a and 1b are most common [10,11], followed by genotypes 2 and 3. Genotype 4 is

more common in Egypt [12], genotype 5 in South Africa [5] and genotype 6 in south east Asia. Changes of genotype from one to the other have also been noted in some studies of intravenous drug users [13, 14]. This has significant treatment implications as responsiveness to treatment depends on the infecting genotype. Mixed infections of more than one genotype in a given patient has been documented [15].This will also have an impact on treatment as the duration differs depending on the infecting genotype.

1.4 Pathogenesis of HCV Related Liver Disease

The lack of animal models and of in vitro culture systems hampers the understanding of the pathogenesis of chronic HCV related liver disease. Hepatocellular injury from HCV is thought to result from host immune mediated responses to infection which may be non-specific such as interferon production or virus specific immune responses [15]. The liver lesions are mainly a result of immune-mediated mechanisms, which are characterized by a type 1 T helper cell response [44].

Acute HCV infection is not clinically manifest in most patients and is most often diagnosed incidentally during routine medical checks for other illnesses. The disease onset is often assumed based on history of exposure to potential risk factors and some patients do not have a history suggestive of an acute hepatitic illness. Only a small proportion of patients (15-20%) are able to clear the acute infection, whereas most patients (80-85%) go on to develop chronic HCV infection [7, 41]. Most of the chronically infected patients have relatively mild disease but 20-25% of them will develop cirrhosis and/ or hepatocellular cancer over the course of several decades [16].

Secretion of cytokines such as interleukin 2, tumour necrosis factor (TNF) α and interferon (IFN) γ by CD4+ T lymphocytes activates antiviral mechanisms that can have a role in clearing the infection. CD 8+ cells might be involved in direct killing of infected cells and might assist viral clearance by secretion of cytokines such as IFNγ and TNF α[12]. The innate immune system also appears to play a role through recognition of pathogen associated membrane patterns via pattern recognition receptors like Toll like proteins. The discovery that NS3/A4 (a non-structural HCV protein) protease can interfere with the activation and nuclear translocation of interferon regulatory factor (IRF) suggested that HCV might interfere with

Toll Like Receptor(TLR) signaling, because IRF3 is an important downstream signal of TLR3, which senses double stranded RNA.

Viral interference with innate immune recognition might contribute to the progressive loss of adaptive response to HCV during early chronic infection, and might provide a rationale for treatment based on the inhibition of the viral protease [7].

Acquiring valid information on the natural history of chronic HCV is made difficult not only by the long duration needed to gather information but also by the fact that there may be accompanying factors that may modify the course such as antiviral treatment, co-infections with Hepatitis B (HBV) and HIV, and concomitant alcohol use [16].Patients infected with HCV usually die from their co-morbid illnesses as opposed to the sequelae of infection with the virus itself.

1.5 Hepatitis C and Diabetes Mellitus

There are millions of people suffering from DM and HCV and, both of them are two common disorders that have the potential to cause devastating long-term complications in a significant number of patients.

HCV mainly targets the liver but it does not cause exclusive liver involvement. It has been associated with a wide range of extrahepatic manifestations including mixed cryoglobulinemia, glomerulonephritis, Porphyria Cutanea Tarda, Sjogren's syndrome and autoimmune thyroiditis among others [17]. Type 11 cryogobulinemia has been the most documented extrahepatic manifestation. The proposed mechanism is an autoimmune phenomenon where there is antigenic cross-reactivity between HCV and antigens encoded by the host genome and formation of immune complexes which are deposited in the different organs. Malignancies like hepatocellular carcinoma and Non-Hodgkins lymphoma are possible complications of chronic HCV infection [32].

With regards to diabetes, a number of mechanisms have been shown to lead to insulin resistance and potentially diabetes mellitus in humans and animals infected with HCV. The potential mechanisms are as follows:

1.5.1 Effects on the Insulin Signal Transduction Pathway

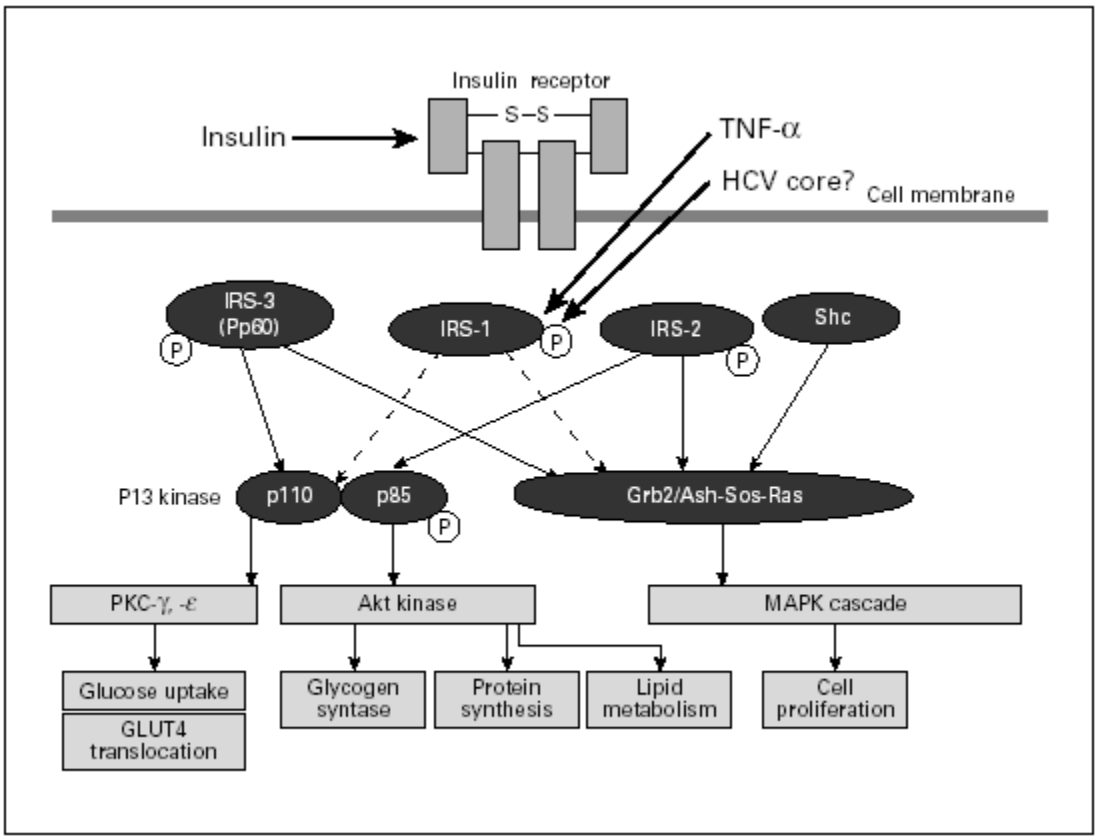
When insulin binds to its receptor, it stimulates intrinsic tyrosine kinase activity, leading to receptor autophosphorylation and recruitment of intracellular signaling molecules such as insulin receptor substrates (IRS). This is followed by a cascade of events finally leading to translocation of glucose transporters (GLUT4) to the cell membrane, resulting in glucose uptake. Activation of other insulin receptor signaling pathways induces glycogen synthesis, protein synthesis, lipogenesis, and regulation of various genes in insulin responsive cells.

HCV consists of structural (core, E1, E2) and non-structural proteins NS2-NS5B [39]. The HCV core protein has been shown to decrease expression of IRS 1 and IRS 2 in human hepatocytes [18]. If the liver does not perceive the insulin signal, it will continue to produce glucose in the fed state leading to marked hyperglycemia and compensatory hyperinsulinemia. Elevated levels of pro-inflammatory cytokines like tumour necrosis factor (TNF) α and interleukin 6 have been demonstrated in patients with chronic HCV infection [41]. HCV also down-regulates insulin receptor substrates 1 and 2 through up-regulation of

suppressor of cytokine signaling 3 (SOCS3) and TNF α [18.19]. IRS 1 and 2 are important for insulin mediated glucose uptake and their down-regulation results in insulin resistance and possibly overt diabetes (Figure 2).

One of the features of the metabolic syndrome is insulin resistance in addition to hypertension, dyslipidemia and central obesity [20]. Patients chronically infected with HCV may develop insulin resistance without the other classical features of the metabolic syndrome [21]. Insulin resistance has been shown in mice transgenic for the HCV core gene and after being fed a high-fat diet, these mice developed overt diabetes [22].These findings suggest that body weight gain may trigger the process leading to overt diabetes. The insulin resistance leads to hepatic steatosis and promotes fibrosis progression in a genotype-dependent manner. Genotype 3 causes more extensive steatosis but lower insulin resistance than the other genotypes [23]. With genotype 1, the steatosis seems to be an expression of the metabolic syndrome and the increased prevalence of diabetes in HCV has been shown to be predominantly among genotype 1 and 2-infected subjects [23,24].

Figure 2: HCV Induced Insulin Resistance



Borrowed from Kazuhiko Koike: Hepatitis C virus infection can present with metabolic disease by inducing insulin resistance. A proposed mechanism of insulin resistance in HCV infection [25]: HCV itself or elevated levels of cytokines such as TNF α may inhibit tyrosine phosphorylation of IRS-1 in the liver, suppress intracellular transduction of insulin signal and lead to insulin resistance. PKC=Protein Kinase C, MAPK=Mitogen-activated protein kinase

1.5.2 Effects on the Pancreas:

There is evidence of pancreatic beta cell dysfunction in patients with HCV. Involvement of the pancreas by the virus results in morphological changes within the gland and altered islet cell function [26]. The morphological changes are accompanied by reduced in vitro glucose-stimulated insulin release, thus the virus is thought to be directly cytopathic to the pancreas. However an increase in apoptosis in the pancreas has not been demonstrated.

1.6 Prevalence of Glucose Abnormalities in Patients with HCV

The liver plays a key role in blood glucose control and thus a variety of acute and chronic liver diseases causing cirrhosis are associated with glucose abnormalities. However, the association of HCV infection and diabetes is higher than that expected by chance alone. Insulin resistance as determined by the homeostasis model assessment (HOMA-IR) has been demonstrated in chronic HCV patients lacking any evidence of liver injury[23, 25].

In 1994, H Gray et al [27] assessed 200 British patients with type 2 diabetes and abnormal liver function tests, recruited from the United Kingdom Prospective Study of Diabetes (UKPDS) for Hepatitis C antibody positivity. The participants were divided into 100 white Caucasians, 50 Afro-Caribbeans and 50 Asians. The findings indicated a high prevalence of HCV infection especially in Afro-Caribbeans (28%), followed by Caucasians (12%) and Asians (8%).This study suggested that abnormal liver functions in diabetic patients should not just be attributed to diabetes, and that HCV must be considered as a cause.

In another study, Rafael Simo et al [28] compared a total of 176 diabetic patients to 6,172 blood donors, matched by recognized risk factors to acquire HCV infection. They found a higher prevalence of HCV in diabetic patients in comparison with blood donors (11. 5% vs. 2.5%; $P<0.001$). They did not detect any particular epidemiological factor for HCV infection and most of the anti-HCV positive patients presented with abnormal liver function tests.

The question however, is whether HCV infection itself leads to diabetes or whether the HCV positive diabetic patients have inherent risk factors to develop diabetes despite being infected. Diabetic patients have a risk of being infected with HCV because of the frequent hospitalizations and possible exposure to unsterile needles when their fingers are pricked to test blood glucose levels.

A prospective study done by Mehta et al [29] found an increased incidence of type 2 diabetes in persons with recognized diabetes risk factors and HCV infection compared with those with similar diabetes risk factors but not HCV infection . They suggest that pre-existing HCV infection may increase the risk of type 2 diabetes in persons with recognized diabetes risk factors.

As glucose abnormalities can be seen in patients with any chronic liver disease, Chen et al [30] investigated the seroprevalence of HBV and HCV infections in type 2 diabetic patients. The diabetic patients were compared with a control group of subjects who came for medical check-ups. No significant difference was found between the type 2 diabetic patients and the control group for seropositivity of HBV surface antigen p=0.441. Anti-

HCV seropositivity was detected in 6.8% of patients and 2.6% of the control subjects (OR=2.87, 95% CI: 1.51-5.46; P<0.001).

2. **Motivation for the Study**

Hepatitis C is a virus with a broad range of clinical manifestations, some of which are fatal like hepatocellular carcinoma. High prevalence rates have been noted in some parts of Africa whereas South Africa does not seem to be as much affected. South Africa however has high rates of other blood transmissible infections like HIV and hepatitis B, which is of concern as more and more cases of viral co-infections are being diagnosed.

Diabetes is a common disease which carries significant morbidity and mortality. Insulin resistance due to a number of factors may progress to type 2 diabetes and that has been demonstrated in studies of animals and humans infected with HCV[18-22]. With newer therapies available and more being developed, HCV is one of the viruses that can potentially be fully eradicated. Treating HCV could theoretically reduce the number of patients developing HCV related diabetes mellitus and all the other extrahepatic manifestations associated with it.

3. Central Chapters

3.1 Study Methods and Design

For this study, patients attending the diabetes clinic at the Charlotte Maxeke Johannesburg Academic (CMJA) Hospital participated. Both type 1 and type 2 patients over the age of 18 years were included and consecutively tested for HCV. The test kit used to test for the presence of HCV antibodies was the HCV Rapid Test device (Schering Plough (PTY) LTD), which is a point-of-care chromatographic immunoassay for the qualitative detection of antibodies to HCV in whole blood, serum or plasma. The sensitivity of the test is 98.5% and its specificity 97.3%. Its limitations were that it only provided qualitative detection of antibodies to HCV and secondly, a negative result did not preclude the possibility of HCV secondary to actual absence or inadequate quantities of such antibodies. The test was a finger prick test where a drop of blood was applied onto the test kit and results were read after a ten minute waiting period.

Informed written consent (Appendix 2) was obtained from all participants before proceeding with the test. The patients were requested to complete a

questionnaire (Appendix 3) with the investigator to obtain details about their demographics, diabetes, body weight, height and exposure to recognized risk factors for HCV infection. The test results for the HCV were recorded on the questionnaire and patients were also informed about their results. The study population was made up of black, white, people of mixed and Asian descent (including Indians and Chinese). The information obtained was captured onto a spreadsheet using Microsoft Access®.

The seroprevalence of HCV from the diabetic group was compared with that of the general population, which was made up of first-time, non-diabetic blood donors. This group, supplied by the South African National Blood Services (SANBS), donated blood from January 2007-December 2007 and they were divided into the black, white, mixed descent and Asian racial groups. The donated blood is routinely screened for blood- borne diseases and those found to be infected are rejected from the donor pool. The test used by the SANBS to screen for HCV is the Polymerase Chain Reaction (PCR).

Inclusion Criteria

Age> 18 years

Exclusion criteria

Presence of diabetes in the blood donor group

3.2 Statistical Analysis

The data were captured using ACCESS database and analysis was done using STATA 9.0 Intercooled. Descriptive statistics for categorical variables was done using frequency distribution tables, reporting the frequencies and percentages. Continuous variables were described by mean values and standard deviations.

The prevalence of HCV for the diabetic group as well as the donor group was computed. The odds ratios of HCV for the diabetes group versus the donor group were computed with a 95% confidence interval. Further analysis on risk factors was done using Pearson's Chi square and where

appropriate the Fischer's Exact Test. Statistical significance was ascertained at the 5% level.

Further analysis based on HCV PCR positivity between the diabetic group and the blood donor group was performed using the chi square test. A confidence interval of 95% with α=5% was chosen.

3.3 Ethical Clearance

Ethical clearance for the study was obtained from the University of the Witwatersrand's Human Research Ethics Committee and is attached in the appendix 4.

4. RESULTS

4.1 Clinical Characteristics of the Diabetic Patients: Table 1

Diabetes Mellitus		Type 1	Type 2
Patient number	N	73	443
Race	Black	28	237
	Non-black	45	206
Age	Years	43±15	55±10
Sex	Males	29	179
	Females	44	264
Diabetes Mellitus duration	Years	20±15	9±7
Treatment	Insulin	98.63%	72.1%
	Sulfonylurea	0%	100%
	Biguanides	0.44%	99.56%
Glycated Haemoglobin %	HbA1c	9.2±2	9±2
Risk factors	Blood T/F	6/73(8. 22%)	55/443(12.42%)
	Scarification	6/73(8.22%)	68/443(15.35%)
	Tattoos	3/73(4.11%)	15/443 (3.39%)
	IVDU	0/73 (0%)	1/443 (0.23%)

A total of 516 diabetic patients attending the diabetic clinic at the CMJA Hospital were recruited for the study. This group was made up of 73 type 1 and 443 type 2 patients. Most of the patients were black, 265 versus 251 non-black (whites, mixed descent and Asians). The type 1 patients were younger than the type 2 diabetes patients and they had had diabetes for a longer duration. Our study group composed of a higher number of females (308) and fewer males (208). Almost all the patients with type 1 diabetes required insulin alone. One patient was put on both insulin and a biguanide. The type 2 patients were on both biguanides and sulfonylureas and 72. 1% were insulin requiring due to poorly controlled diabetes on oral hypoglycemics.

The mean glycated haemoglobin (HbA1c), which serves as a marker for glycaemic control, was about 9% for both groups. The recommended level for adults is <7% for macro- and microvascular risk reduction [35] and that level is used to guide treatment decisions.

Blood transfusions and traditional scarifications were the two high risk factors that most of our patients were exposed to (Graph 1). Only one patient from the type 2 group admitted to intravenous drug use. Only 18 patients in total had tattoos.

Figure 3: Risk Factors for HCV in Type 1 and Type 2 DM Patients

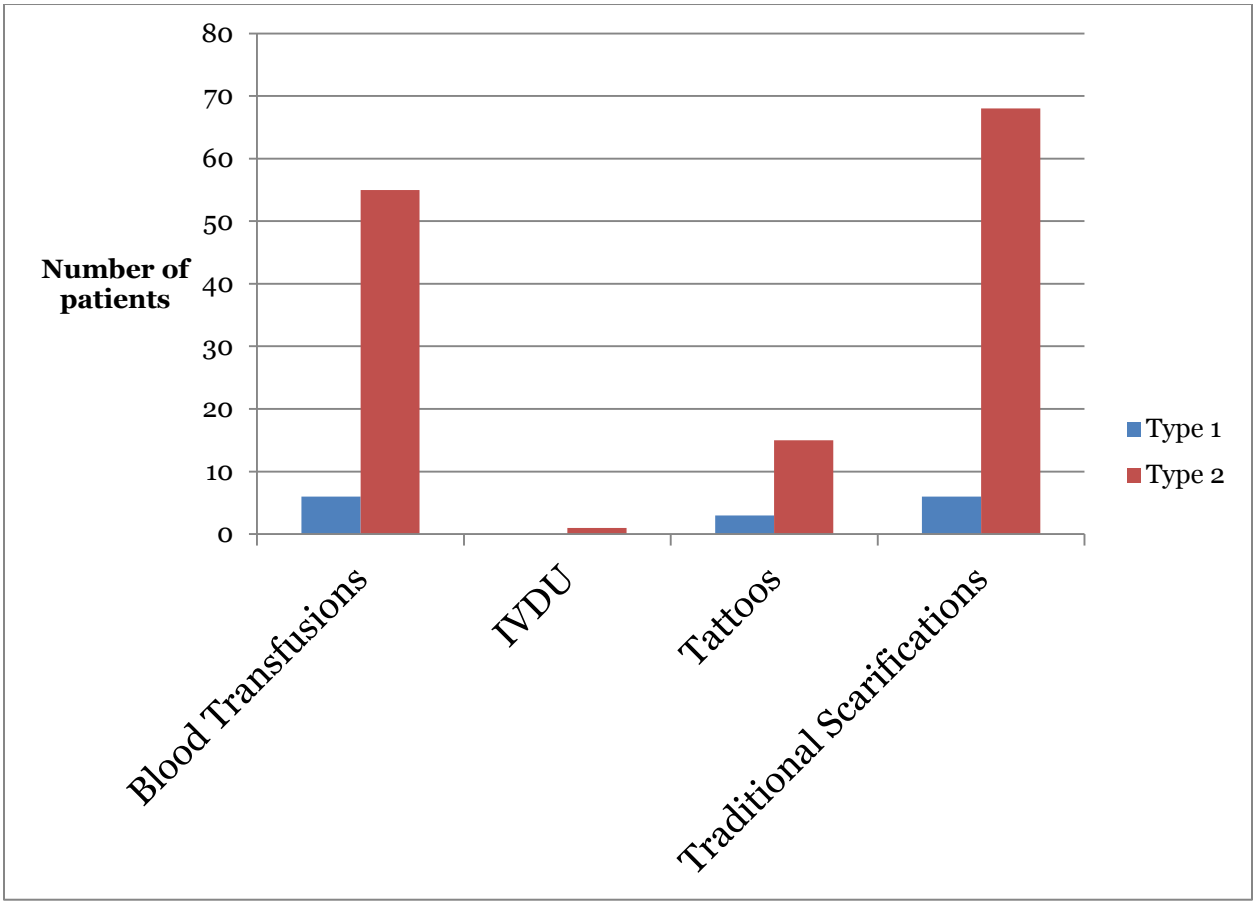


Table 2 compares the demographics of the diabetic patients who tested positive for HCV and those who tested negative. A total of eight patients were anti-HCV positive and that included one patient with type 1 DM and seven with type 2. There was no statistically significant difference in the patients' ages, the type of diabetes nor the duration of the diabetes. None of the patients who were positive had tattoos or history of intravenous drug use but 12.5% had experienced traditional scarifications and 12.5% had received blood transfusions.

TABLE 2: Demographic Comparisons between the Diabetic Patients who are anti-HCV positive and anti-HCV negative

		Anti HCV positive	Anti-HCV negative	*P value*
Patients	N	8	508	
Age	Years	55±16	53±12	N/S
Type of DM	Type 1	1.37%	98.63%	N/S
	Type 2	1.5%	98.42%	N/S
Duration of DM	Years	8±5	11±9	N/S
Risk factors	Blood T/F	12.5%	11.81%	N/S
	Scarification	12.5%	14.37%	N/S
	Tattoos	0%	3.54%	N/S
	IVdrug use	0%	0.2%	N/S

Exposure to recognized risk factors for HCV did not increase the rates of HCV infection.

We then compared our diabetic patients to the blood donor group for prevalence of HCV. We had a total of 35 194 people who donated blood in 2007. This group was composed of 19 181 males and 16 013 females. There were 2 778 Asians, 3 165 black Africans, 2 011 persons of mixed descent and 27 240 white people. There was an age difference between the diabetic patients and the blood donors but this was not statistically significant. There were 8 HCV positive patients from the blood donor group as well as 8 from the diabetes group.

TABLE 3: Comparison between the diabetic patients and the blood donors on antibody testing

	Blood donors	**Diabetic group**	***P value***
n	35194	516	
Age in years	40±13.02	55±5	N/S
Sex			
Males	19181	208	
Females	16013	308	
HCV Antibody Positive	**8/35194(0.02%)**	**8/516(1. 55%)**	**<0.0001**

Our study demonstrated a higher prevalence of HCV in diabetes patients than in the blood donor group and that difference is statistically highly significant (1.55% vs. 0.02%; *P*<0.0001; odds ratio 69; 95%CI 22.5-212.4). As our patients had been tested for HCV infection with the use of a rapid finger prick test and not PCR, we invited them to our liver clinic for HCV PCR testing. Six out of the eight patients presented to the clinic. Two of the patients did not attend the clinic and, numerous attempts to contact them were unsuccessful. Five of the six patients were positive for HCV PCR and one was negative. This showed that at least one of our patients had a false positive result. Genotypes identified in four of the five patients were 1, 4a, and two patients with 5a.

TABLE 3:Comparison between the diabetic patients and the blood donors on HCV PCR

	Blood donors	**Diabetic group**	***P value***
n	35194	516	
Age in years	40±13.02	55±5	N/S
Sex **M** **F**	 19181 16013	 208 308	
HCV PCR Positive	8/35194(0.02%)	5/516(0.97%)	0.005

5. DISCUSSION

HCV was found in 1.55% of our patients, a prevalence which is some 7.5 fold greater than for the general South African population at large (1.55% vs. 0.02%; P <0.001; odds ratio 69;95% CI; 22-212). On PCR testing, the prevalence of HCV in our diabetic patients was 0.97%. Our results support the recent evidence of an association between HCV and increased risk of developing diabetes. Simo et al[28] found HCV infection in 11.5% of diabetic patients in comparison with 2.5% in blood donors. Gray et al[27] also found a higher prevalence of HCV in diabetic patients, particularly so in Afro-Caribbean participants. Our patient population was exposed to all the known risk factors for developing HCV, but previous exposure to these did not appear to significantly increase the rate of infection among them. Some of the patients who tested negative had used IV drugs in the past and even though that has been described as the commonest route of infection elsewhere [6], it was not the case in our setting. We hypothesize that the reasons for that difference is because IV drug use may not be as common a practice in RSA as it is in other countries or that there was exclusive use of injection paraphernalia in our subjects. Blood transfusions and traditional scarifications are the only two risk factors that our patients who tested positive for HCV were exposed to. Two of the type 2 diabetic patients had

both the blood transfusion and traditional scarifications and they were exposed to these risk factors before they developed diabetes. However, there was no statistically significant difference with regards to risk factors between those infected and those not (12.5% vs. 11.81% for transfusions and 12.5% vs. 14.37% for scarifications). The blood donor group could not be compared to the diabetic with regards to risk factors for acquiring HCV as that data was not available from the blood bank records provided. The prevalence of HCV infection is low in South Africa [5], and that is also supported by the finding of a 0.02% prevalence rate in our blood donor group. Our diabetic patients were generally older than the blood donor group, but that difference was not statistically significant.

Studies have shown an age effect on the chronicity of HCV with rates of 30% in subjects below the age of 20 and 76% in those older than 20 years [34]. There was a higher prevalence of HCV infection in our type 2 diabetic group as compared to the type 1 diabetics, this was however not statistically significant. The duration of diabetes was almost similar in the two groups and both groups had had previous exposure to high risk behaviour for acquiring HCV. At this point in time, nothing points towards any particular

risk factor as being the cause of increased HCV prevalence in our diabetes patients.

The natural progression of chronic HCV is that infected people only manifest clinical disease years after exposure to the virus [8]. When recruited to participate in the study, the diabetic patients had no prior history of liver disease or current symptoms to suggest liver disease. A number of host and viral factors for accelerated disease progression have been identified and, amongst them are the presence of steatosis, insulin resistance, high fasting glucose and hyperinsulinemia [25, 45]. These factors are often present in patients with DM and may predispose HCV infected patients to accelerated fibrosis and eventually cirrhosis. The risk of developing HCC for a patient with HCV-related cirrhosis is approximately 2-6% per year and HCC risk increases to 17-fold in HCV infected patients compared to HCV-negative subjects [46].

This study cannot prove that HCV itself had any causative role in the development of diabetes mellitus. However, meta-analysis of studies reporting Hazards Ratios (HR) demonstrated that HCV infection significantly increases the risk of developing diabetes[11]. Numerous other studies have shown that chronic HCV infection is associated with insulin

resistance which can progress to overt diabetes. The proposed mechanisms include amongst others, activation pro-inflammatory mediators [18-19] and a direct involvement of the pancreas by the virus itself [26].

HCV infected patients have been shown to develop insulin resistance in the absence of other features of the metabolic syndrome. Clinical variables such as age, black ethnicity, male gender, cirrhosis or advanced histologic stage [22, 27, 29, 36] are associated with development of diabetes in patients with chronic HCV. What is evident is that most of our patients had other risk factors for diabetes, for example black ethnicity and advanced age. Only one patient from the group of patients who tested positive agreed to have a liver biopsy. She was a 68 year old black lady whose histology showed features of chronic hepatitis with incipient cirrhosis, in keeping with chronic HCV infection.

The study by Mehta et al [29] was a prospective analysis to examine if persons who acquired type 2 diabetes were more likely to have had antecedent HCV infection. Persons were categorized as low-risk or high-risk for diabetes based on their age and body mass index, factors that appeared to modify the type 2 diabetes-HCV infection incidence estimates. Among those at

high risk for diabetes, persons with HCV infection were more than 11 times as likely to develop diabetes (relative hazard 11,58; 95%CI, 1.39-96.9). Among those at low risk, no increased incidence of diabetes was detected among HCV infected persons (relative hazard, 0.48; 95% CI 0.05-4.40).From this study it appears that a combination of the known diabetes risk factors and infection with HCV (host and viral mediated factors respectively) places patients at a greater risk of diabetes.

The mean body mass index (BMI) of our patients (not shown in the report) was 30.1 kg/m^2. Even though these patients had an inherent risk factor for diabetes shown by a high BMI, we believe that HCV had a role to play in the development of their diabetes. Wang et al [36] showed in a community-wide and population based cohort study, after adjusting for established risk factors for diabetes- age, gender, educational level, body mass index, smoking, and alcohol consumption- in a multivariate Cox proportional hazards analysis that persons with HCV infection had a significantly (70%) higher incidence of diabetes than those without HCV infection. This finding was consistent with other studies that showed that HCV infection is highly associated with diabetes.

6. Limitations of the Study

1. We were not able to properly compare the diabetic group and the donor group with regards recognized risk factors for acquiring HCV as that data was not available.

2. We were not able to perform confirmatory HCV PCRs in two of the patients who tested positive on the rapid antibody test and this has affected our final result.

3. The confidence interval of the odds ratio for acquiring Hepatitis C infection among our diabetic cohort was wide and is likely the consequence of a small sample size. Greater precision should come from studying larger numbers.

7. Conclusion

The study demonstrates a higher prevalence of HCV in our diabetic patients in comparison to the general population. It is however not possible to draw firm conclusions of whether HCV is a risk factor for diabetes. To do so we would need to follow up non-diabetic persons infected with HCV over a period of time and see whether they develop diabetes or not. In the interim, patients with chronic HCV should be monitored closely for abnormalities of glucose homeostasis. Likewise, patients with diabetes who develop abnormalities in liver function tests should be tested for infection with HCV.

Finally, a study by Veldt et al [43] showed an increased risk of hepatocellular carcinoma in patients with HCV cirrhosis and diabetes. This highlights the need for surveillance for HCC in these patients and the need for education of the population at large about avoiding high risk behaviours for acquiring HCV and developing diabetes, as it might be possible to reduce the incidence of this aggressive malignancy by such means.

APPENDICES

Appendix 1

RESEARCH PROTOCOL

SEROPREVALENCE OF HEPATITIS C IN DIABETIC PATIENTS IN AN URBAN SOUTH AFRICAN SETTING

Researcher: Dr M E Seabi

Name of Degree: Mmed (Internal Medicine)

Student number: 0317222J

Supervisors

1. Professor E Song

FCP (SA), FRCP (London)

Associate Professor, Principal Specialist

Department of Medicine

Wits medical school

Area 550, Johannesburg Hospital

2. Dr P H Barrow

FCP (SA), Certificate of gastroenterology.

Consultant in Medical gastroenterology department.

Introduction

Hepatitis C was discovered in 1989 and affects approximately 170million people worldwide(1,2). Chronic Hepatitis C is a progressive condition and the spectrum of liver disease range from chronic inflammation, cirrhosis to hepatocellular carcinoma.

Extrahepatic manifestations of chronic hepatitis C are clinically evident in nearly 40% of patients(2). Mixed cryoglobulinaemia, membranoproliferative glomerulonephritis and porphyria cutanea tarda, Sjogren's syndrome, lymphoproliferative disorders and neuropathies are strongly associated with hepatitis C infection. The pathophysiologic basis for most of these syndromes seems immunologic(3).

More recently, the association of insulin resistance and diabetes mellitus with chronic hepatitis C has been demonstrated. Data from several studies indicate that the prevalence of diabetes mellitus is high among patients with chronic hepatitis C(2,4,5,6).

Chen et al did a study in April 2006 to investigate the seroprevalence of hepatitis B and C virus infections in type 2 diabetes mellitus patients. No significant difference was found between type 2 diabetes patients and the control group for seropositivity for HBsAg, but the rate of seropositive anti-HCV was 2,8 times higher in type 2 diabetes patients than in non-diabetic control subjects(6).

Allison et al in a retrospective study showed that the prevalence of diabetes in patients with chronic hepatitis C was 50% compared with 9% of the control population (7).

Several clinical variables are associated with an increased prevalence of diabetes in patients with chronic hepatitis C. Some host factors include age, obesity, male gender, black ethnicity, alcohol consumption and a family history of diabetes(8). Hepatitis C is thought to cause diabetes through a variety of mechanisms including:

1.It induces hepatic steatosis and increases tumour necrosis factor alpha, both resulting in the development of insulin resistance and subsequent type 2 diabetes.(1,8)

2. Hepatitis C encoded proteins might alter insulin signaling thus leading to impaired insulin sensitivity and glycemic dysregulation (9).

3. Pancreatic beta cell dysfunction (8).

Host and viral factors therefore may work synergistically to effect histopathologic change in the form of steatosis, inflammation, and fibrosis development. Once this occurs, data suggest that progression to diabetes may occur in patients with underlying genetic susceptibility(8).

Study Objectives

1. To determine the seroprevalence of Hepatitis C in patients attending the diabetes clinic

at Johannesburg Hospital.

2. To compare the prevalence of hepatitis C in our type 2 diabetics with that in the case controls (healthy blood donors)

3.The patients that test positive for hepatitis C will be assessed to see if they have any of the risk factors that are associated with the development of diabetes e.g.: obesity, alcohol consumption.

4. The information will also be used to determine if previous exposure to high risk behavior for hepatitis e.g.: traditional scarifications, blood transfusions etc are associated with earlier onset of diabetes.

5.The clinical records of those who test positive for hepatitis C will be used to see if the presence of diabetes is associated with more severe liver disease or not. Further surveillance for those patients will be at done at the liver clinic and there we will do a blood test for hepatitis C RNA .

Methods

All patients who attend the diabetes clinic at the Johannesburg hospital will be offered HCV screening. The sample will include all patients who are 18yrs or older, from the black African, white, mixed descent and Asian racial groups. To control for selection bias, randomization will be done. Patients will be divided into the different racial groups and every third patient from the each group will be included into the study.

The seropositivity for hepatitis C will be determined by the use of testing kits which detect the presence of hepatitis C antibodies. The names of the test kits are Pegatest One step HCV test kit provided by Roche Products (pty) ltd and the hepatitis C testing kit from Schering Plough (pty) ltd . Capillary blood will be obtained by the finger prick method and a drop placed onto the testing kit. The result will be available within minutes. It is a rapid test and will be performed at the same time that the finger prick test for checking blood glucose is done.

The test kit comes with easy instructions for interpretation but has these limitations:

1. Only provides qualitative detection of antibodies to HCV.
2. A negative result does not preclude the possibility of HCV infection secondary to actual absence or inadequate quantities of such antibodies.

Specificity and Sensitivity

Sensitivity – 98.5%

Specificity – 97.3%

All patients who undergo screening will have the results interpreted by diabetic clinic doctors and a HCV health questionnaire will be completed (see attached health questionnaire).

For case controls, healthy blood donors will be used. Records will be obtained from the South African blood transfusion services providing age, sex and race matched controls to compare with our study group. The age range from our case control will be +-5yrs.

Data analysis

A database will be set up to evaluate all the data obtained. Comparison will be made between the different race groups, type1 and type 2 diabetes and an age, sex and race matched group of healthy blood donors provided by Dr Crookes from the South African National Blood services. The age range from our case control will be ±5yrs.

Statistical analysis will be performed using the Mann-Whitney test for comparison of continuous variables between groups and corrected chi-squared method for qualitative data where appropriate. A two-tailed P-value of <0.05 will be considered to be statistically significant.

Ethics

The patients participating in the study will be asked to sign an informed consent form explaining exactly what the procedure entails. Permission to carry out the study will also be requested from the head of the Department

of Medicine and the hospital superintendent. Participation will be on a voluntary basis and all the information captured will only be made known to the Wits University and the scientific community only. The study will be submitted to the ethics committee in August 2006.

Timing

The study will commence as soon as ethics approval has been obtained. The duration will be for a period 6 months.

Funding

The hepatitis C testing kits will be provided by pharmaceutical companies. Roche products (pty)ltd will provide the Pegatest one step HCV test kit and Schering Plough(pty)ltd will provide the Hepatitis C testing kit.

Appendix 2

PATIENT INFORMATION AND INFORMED CONSENT:

Hello. My name is Dr Seabi and I work at the Department of Internal Medicine at the Johannesburg Hospital.

I am doing a project to determine how many patients with Diabetes have evidence of exposure to a germ called “Hepatitis C virus”. The virus affects the liver and can cause ongoing inflammation, scarring or cancer of the liver. There have been reports that Hepatitis C can cause Diabetes.

To carry out this study I will have to collect a drop of blood from you by using the “finger prick method”. This is the same method used routinely to check your blood glucose level at the clinic or at home. The blood collected will be applied on to the Hepatitis C testing kit to check if you are infected with the virus or not.

The procedure will be carried out under sterile conditions but may result in some discomfort, pain or bleeding at the puncture site.

It is up to you to decide whether or not to take part in this study. Whatever you decide, the standard of your future medical care will not be affected. Even if you do decide to take part, you are free to withdraw from the study at any time. Please note that the results of this study will be completely anonymous and your confidentiality will be maintained at all times.

Those who test positive for Hepatitis C will be offered further surveillance at the liver clinic where their progress will be monitored.

You will not be paid to participate in this study and do so of your own free will

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I have been fully informed about the purpose and methods of this study and, in signing, agree to participate in the research.

Name.....Signature.....Date.....

Doctor.....Signature.....Date.....

Appendix 3

HEALTH QUESTIONNAIRE

Please complete the following questionnaire

Race : Black:☐ White: ☐ Indian: ☐ Other: ☐

Background: Rural: ☐ Urban: ☐ Rural/Urban: ☐

Height: ……meters **Weight**: ……Kg

Type of Diabetes: Type 1: ☐ Type 2: ☐ Unspecified: ☐

Duration of Diabetes:……years

Diabetes Medication (Please list):

Oral:…… Injectable……

…… ……

…… ……

…… ……

Most recent HbA1c: .....%

Alcohol Consumption: Beer: ☐ Wine: ☐ Spirits: ☐
Other: ☐

Units per week: <7: ☐ 8-14: ☐ 5-21: ☐ >21: ☐
(<1/day) (1-2/day) (2-3/day) (>3/day)

Have you had any of the following procedures?

1. Blood transfusions: Yes: ☐ No: ☐
   (If yes: ? before 1990: Yes: ☐ No: ☐)
2. Traditional scarifications: Yes: ☐ No: ☐
3. Tattoos: Yes: ☐ No: ☐
4. Intravenous drug use: Yes: ☐ No: ☐

Hepatitis C result :

Positive: ☐ Negative: ☐

Appendix 4

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Seabi

CLEARANCE CERTIFICATE	**PROTOCOL NUMBER M060802**
PROJECT	Seroprevalence of Hepatitis C in Diabetic Patients in Urban South african Setting
INVESTIGATORS	Dr ME Seabi
DEPARTMENT	Department of Medicine
DATE CONSIDERED	06.08.25
DECISION OF THE COMMITTEE*	Approved unconditionally

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 06.11.09 **CHAIRPERSON** .....
(Professors PE Cleaton-Jones, A Dhai, M Vorster, C Feldman, A Woodiwiss)

*Guidelines for written 'informed consent' attached where applicable

cc: Supervisor : Prof E Song

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10005, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

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